

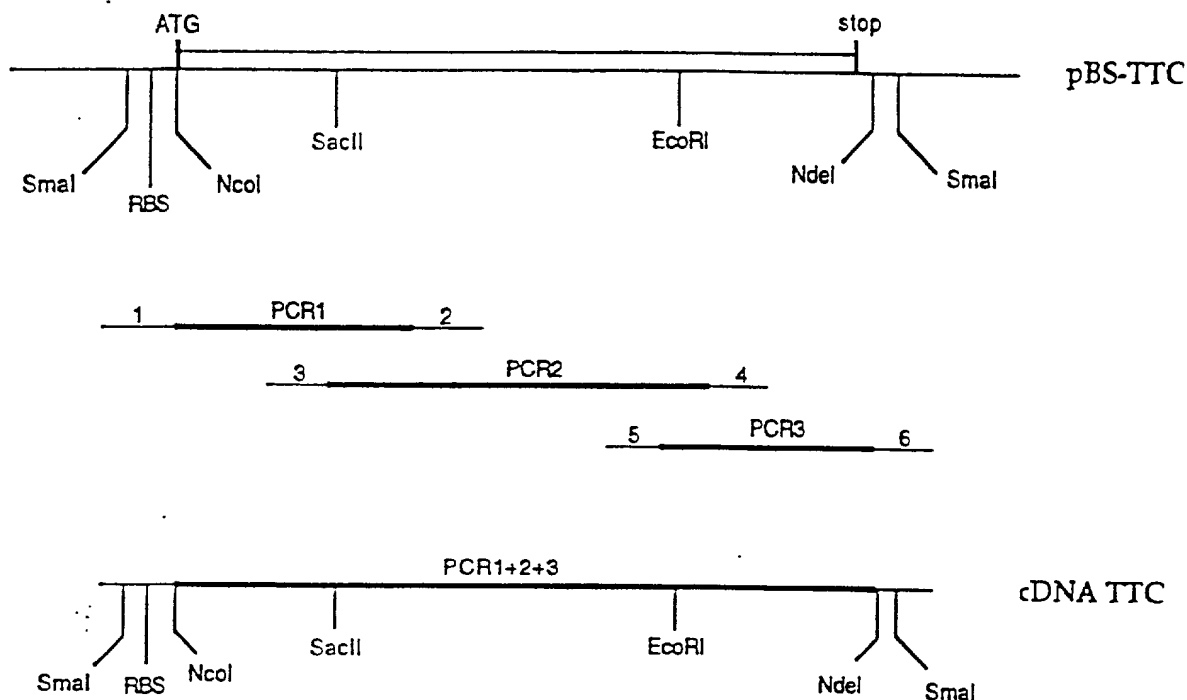
SK-TTC -> Genes

DNA sequence 1600 h.p. ggaaacagctat ... gtctgtttacaa linear



00816467-062901

1 ggaaacagctatgaccatgattacgccaagctcgaaattacccctcactaaagggacaaaagctggagctcggtaccgg 80
81 ggccacc ATG GTT TTT TCA ACA CCA ATT CCA TTT TCT TAT TCT AAA AAT CTG GAT TGT TGG 141
1 M V F S T P I P F S Y S K N L D C W 18
142 GTT GAT AAT GAA GAA GAT ATA GAT GTT ATA TTA AAA AAG AGT ACA ATT TTA AAT TTA GAT 201
19 V D N E E D I D V I L K K S T I L N L D 38
202 ATT AAT AAT GAT ATT ATA TCA GAT ATA TCT GGG TTT AAT TCA TCT GTA ATA ACA TAT CCA 261
39 I N N D I I S D I S G F N S S V I T Y P 58
262 GAT GCT CAA TTG GTG CCC GGA ATA AAT GGC AAA GCA ATA CAT TTA GTA AAC AAT GAA TCT 321
59 D A Q L V P G I N G K A I H L V N N E S 78
322 TCT GAA GTT ATA GTG CAT AAA GCT ATG GAT ATT GAA TAT AAT GAT ATG TTT AAT AAT TTT 381
79 S E V I V H K A M D I E Y N D M F N N F 98
382 ACC GTT AGC TTT TGG TTG AGG GTT CCT AAA GTA TCT GCT AGT CAT TTA GAA CAA TAT GGC 441
99 T V S F W L R V P K V S A S H L E Q Y G 118
442 ACA AAT GAG TAT TCA ATA ATT AGC TCT ATG AAA AAA CAT AGT CTA TCA ATA GGA TCT GGT 501
119 T N E Y S I I S S M K K H S L S I G S G 138
502 TGG AGT GTA TCA CTT AAA GGT AAT AAC TTA ATA TGG ACT TTA AAA GAT TCC GCG GGA GAA 561
139 W S V S L K G N N L I W T L K D S A G E 158
562 GTT AGA CAA ATA ACT TTT AGG GAT TTA CCT GAT AAA TTT AAT GCT TAT TTA GCA AAT AAA 621
159 V R Q I T F R D L P D K F N A Y L A N K 178
622 TGG GTT TTT ATA ACT ATT ACT AAT GAT AGA TTA TCT TCT GCT AAT TTG TAT ATA AAT GGA 681
179 W V F I T I T N D R L S S A N L Y I N G 198
682 GTA CTT ATG GGA AGT GCA GAA ATT ACT GGT TTA GGA GCT ATT AGA GAG GAT AAT AAT ATA 741
199 V L H G S K E I T G L G A I R E D N N I 218
742 ACA TTA AAA CTA GAT AGA TGT AAT AAT AAT AAT CAA TAC GTT TCT ATT GAT AAA TTT AGG 801
219 T L K L D R C N N N N Q Y V S I D K F R 238
802 ATA TTT TGC AAA GCA TTA AAT CCA AAA GAG ATT GAA AAA TTA TAC ACA AGT TAT TTA TCT 861
239 I F C K A L N P K E I E K L Y T S Y L S 258
862 ATA ACC TTT TTA AGA GAC TTC TGG GGA AAC CCT TTA CGA TAT GAT ACA GAA TAT TAT TTA 921
259 I T F L R D P W G N P L R Y D T E Y Y L 278
922 ATA CCA GTA GCT TCT AGT TCT AAA GAT GTT CAA TTG AAA AAT ATA ACA GAT TAT ATG TAT 981
279 I P V A S S S K D V Q L K N I T D Y M Y 298
982 TTG ACA AAT GCG CCA TCG TAT ACT AAC GGA AAA TTG AAT ATA TAT TAT AGA AGG TTA TAT 1041
299 L T N A P S Y T N G K L N I Y Y R R L Y 318
1042 AAT GGA CTA AAA TTT ATT ATA AAA AGA TAT ACA CCT AAT AAT GAA ATA GAT TCT TTT GTT 1101
319 N G L K F I I K R Y T P N N E I D S F V 338
1102 AAA TCA GGT GAT TTT ATT AAA TTA TAT GTA TCA TAT AAC AAT AAT GAG CAC ATT GTA GGT 1161
339 K S G D F I K L Y V S Y N N N E H I V G 358
1162 TAT CCG AAA GAT GGA AAT GCC TTT AAT AAT CTT GAT AGA ATT CTA AGA GTA GGT TAT AAT 1221
359 Y P K D G N A F N N L D R I L R V G Y N 378
1222 GCC CCA GGT ATC CCT CTT TAT AAA AAA ATG GAA GCA GTA AAA TTG CGT GAT TTA AAA ACC 1281
379 A P G I P L Y K K M E A V K L R D L K T 398
1282 TAT TCT GTA CAA CTT AAA TTA TAT GAT GAT AAA AAT GCA TCT TTA GGA CTA GTA GGT ACC 1341
399 Y S V Q L K L Y D D K N A S L G L V G T 418
1342 CAT AAT GGT CAA ATA GGC AAC GAT CCA AAT AGG GAT ATA TTA ATT GCA AGC AAC TGG TAC 1401
419 H N G Q I G N D P N R D I L I A S N W Y 438
1402 TTT AAT CAT TTA AAA GAT AAA ATT TTA GGA TGT GAT TGG TAC TTT GTA CCT ACA GAT GAG 1461
439 F N H L K D K I L G C D W Y F V P T D E 458
1462 GGA TGG ACA AAT GAT TAA acagattgatattgttcatgacatatgccccggatcctctagagtcgacctcgaggy 1535
459 G W T N D * (SEQ ID NO: 2) 464
1536 ggggccccgtaccacaattcgccctatagtgtgtgtattacaattcactggcgtgtttttacaa (SEQ ID NO: 1) 1600



TTC cDNA isolation:

The TTC cDNA was isolated from a *Clostridium Tetani* strain using Polymerase Chain Reaction. We used a three times PCR to generate three overlapping fragments respectively of 465 bp (PCR1; primer 1: 5'-CCC CCC GGG CCA CCA TGG TTT TTT CAA CAC CAA TTC CAT TTT CTT ATT C-3' & primer 2: 5'-CTA AAC CAG TAA TTT CTG-3'), of 648 bp (PCR2; primer 3: 5'-AAT TAT GGA CTT TAA AAG ATT CCG C-3' & primer 4: 5'-GGC ATT ATA ACC TAC TCT TAG AAT-3') and of 338 bp (PCR3; primer 5: 5'-AAT GCC TTT AAT AAT CTT GAT AGA AAT-3' & primer 6: 5'-CCC CCC GGG CAT ATG TCA TGA ACA TAT CAA TCT GTT TAA TC-3'), and each fragment was sequentially cloned into pBluescript KS+ to produce plasmid pBS-TTC. The upstream primer 1 contained the Ribosome Binding Site (RBS) and translation initiation signals.

FIGURE 2

SK-TTC → Genes

DNA sequence	1600 b.p.	ggaacagctat ... gtcgtttacaa	linear
1	ggaacagctatgaccatgattacgccaagctcgaaataaccctcaactaaagggaacaaagctggagctcggtaccg		80
81	gqccacc	ATG GTT TTT TCA ACA CCA ATT CCA TTT TCT TAT TCT AAA AAT CTG GAT TGT TGG	141
1	M	V F S T P I P F S Y S K N L D C W	18
142	GTT GAT AAT GAA GAT ATA GAT GTT ATA TTA AAA AAG AGT ACA ATT TTA AAT TTA GAT		201
19	V D N E E D I I D V I L K K S T I L N L D		38
202	ATT AAT AAT GAT ATT ATA TCA GAT ATA TCT GGC TTT AAT TCA TCT GTA ATA ACA TAT CCA		261
39	I N N D I I S D I S G F N S S V I T Y P		58
262	GAT GCT CAA TTG GTG CCC GGA ATA AAT GGC AAA GCA ATA CAT TTA GTA AAC AAT GAA TCT		321
59	D A Q L V P G I N G K A I H L V N E S		78
322	TCT GAA GTT ATA GTG CAT AAA GCT ATG GAT ATT GAA TAT AAT GAT ATG TTT AAT AAT TTT		381
79	S E V I V H K A M D I E Y N D M F N N F		98
382	ACC GTT AGC TTT TGG TTG AGG GTT CCT AAA GTA TCT GCT AGT CAT TTA GAA CAA TAT GGC		441
99	T V S F W L R V P K V S A S H L E Q Y G		118
442	ACA AAT GAG TAT TCA ATA ATT AGC TCT ATG AAA AAA CAT AGT CTA TCA ATA GGA TCT GGT		501
119	T N E Y S I I S S M K K H S L S I G S G		138
502	TGG AGT GTA TCA CTT AAA GGT AAT AAC TTA ATA TGG ACT TTA AAA GAT TCC GCG GGA GAA		561
139	W S V S L K G N N L I I W T L K D S A G E		158
562	GTT AGA CAA ATA ACT TTT AGG GAT TTA CCT GAT AAA TTT AAT GCT TAT TTA GCA AAT AAA		621
159	V R Q I T F R D L P D K F N A Y L A N K		178
622	TGG GTT TTT ATA ACT ATT ACT AAT GAT AGA TTA TCT TCT GCT AAT TTG TAT ATA AAT GGA		681
179	W V F I T I T N D R L S S A N L Y I N G		198
682	GTA CTT ATG GGA AGT GCA GAA ATT ACT GGT TTA GGA GCT ATT AGA GAG GAT AAT AAT ATA		741
199	V L M G S A E I T G L G A I R E D N N I		218

FIG. 1A

```

742 ACA TTA AAA CTA GAT AGA TGT AAT AAT AAT CAA TAC GTT TCT ATT GAT AAA TTT AGG 801
219 T L K L D R C N N N N Q Y V S I D K F R 238

802 ATA TTT TGC AAA GCA TTA AAT CCA AAA GAG ATT GAA AAA TTA TAC ACA AGT TAT TTA TCT 861
239 I F C K A L N P K E I E K L L Y T S Y L S 258

862 ATA ACC TTT TTA AGA GAC TTC TCG GGA AAC CCT TTA CGA TAT GAT ACA GAA TAT TAT TTA 921
259 I T F L R D F W G N P L R Y D T E Y Y L 278

922 ATA CCA GTA GCT TCT AGT TCT AAT AAA GAT GTT CAA TTG AAA AAT ATA ACA GAT TAT ATG TAT 981
279 I P V A S S S S K D V Q L K N I T D Y M Y 298

982 TTG ACA AAT GCG CCA TCG TAT ACT AAC GGA AAA TTG AAT ATA TAT TAT AGA AGG TTA TAT 1041
299 L T N A P S Y T N G K L L N I Y Y R R L Y 318

1042 AAT GGA CTA AAA TTT ATT ATA AAA AGA TAT ACA CCT AAT AAT AAT GAA ATA GAT TCT TTT GTT 1101
319 N G L K F I I K R Y T P N N E I D S F V 338

1102 AAA TCA GGT GAT TTT ATT AAA TTA TAT GTA TCA TAT AAC AAT AAT AAT GAG CAC ATT GTA GGT 1161
339 K S G D F I K L Y V S Y N N N E H I V G 358

1162 TAT CCG AAA GAT GGA AAT GCC TTT AAT AAT AAT AAT CTT GAT AGA ATT CTA AGA GTA GGT TAT AAT 1221
359 Y P K D G N A F N N L D R I L R V G Y N 378

1222 GCC CCA GGT ATC CCT CTT TAT AAA AAT AAA AAT GAA GCA GTA AAA TTG CGT GAT TTA AAA ACC 1281
379 A P G I P L Y K K M E A V K L R D L K T 398

1282 TAT TCT GTA CAA CTT AAA TTA TAT GAT GAT AAA AAT GCA TCT TTA GGA CTA GTA GGT ACC 1341
399 Y S V Q L K L Y D D K N A S L G L V G T 418

1342 CAT AAT GGT CAA ATA GGC AAC GAT CCA AAT AGG GAT ATA TTA ATT GCA AGC AAC TGG TAC 1401
419 H N G Q I G N D P N R D I L I A S N W Y 438

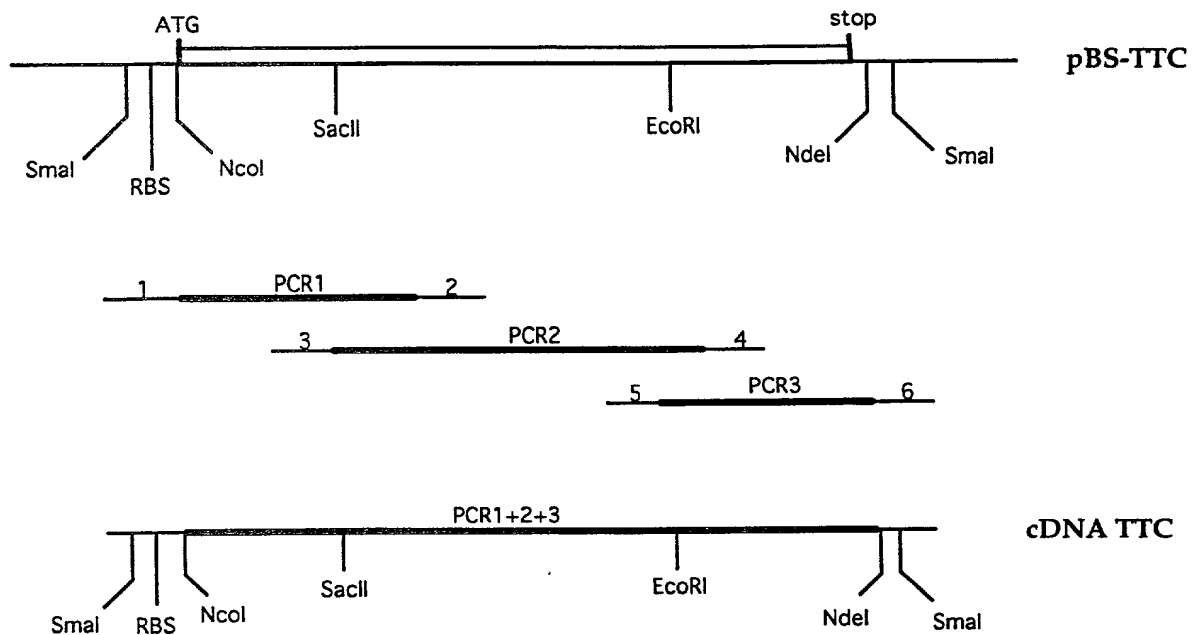
1402 TTT AAT CAT TTA AAA GAT AAA ATT TTA GGA TGT GAT TGG TAC TTT GTA CCT ACA GAT GAG 1461
439 F N H L K D K I L G C D W Y F V P T D E 458

1462 GGA TGG ACA AAT GAT TAA acagattgatattgttcacatattgccccgggatacctctagatcgacctcgagg 1535
459 G W T N D * (SEQ ID NO:2) 464

1536 gggggcccggtaccaccaattcgccctatagttagtcgtattacaattcaactggccgctgctttacaa (SEQ ID NO:1) 1600

```

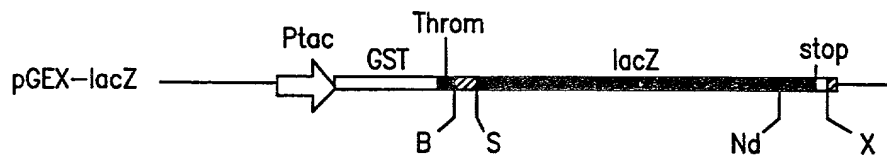
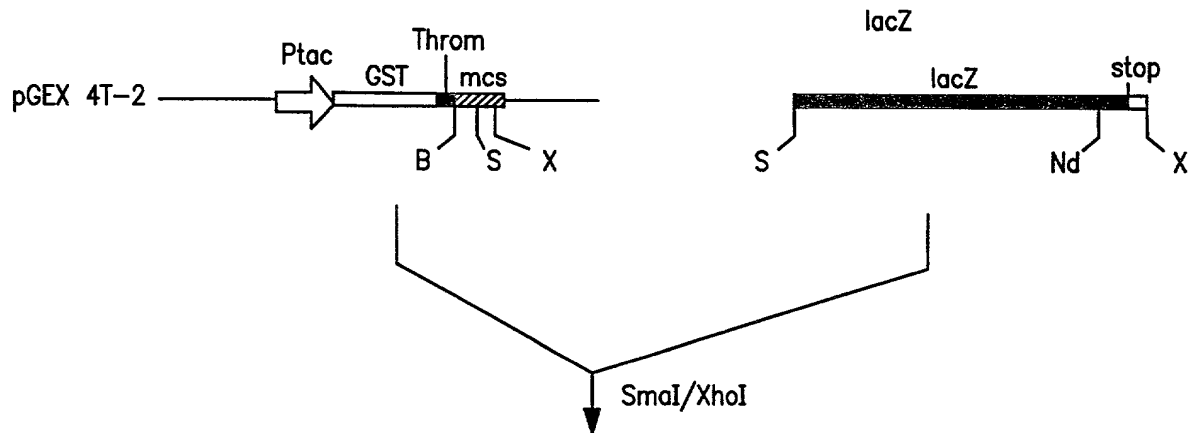
FIG. 1B



TTC cDNA isolation:

The TTC cDNA was isolated from a *Clostridium Tetani* strain using Polymerase Chain Reaction. We used a three times PCR to generate three overlapping fragments respectively of 465 bp (PCR1; primer 1: 5'-CCC CCC GGG CCA CCA TGG TTT TTT CAA CAC CAA TTC CAT TTT CTT ATT C-3' (SEQ ID NO:4) & primer 2: 5'-CTA AAC CAG TAA TTT CTG-3' (SEQ ID NO:5), of 648 bp (PCR2; primer 3: 5'-AAT TAT GGA CTT TAA AAG ATT CCG C-3' (SEQ ID NO:6) & primer 4: 5'-GGC ATT ATA ACC TAC TCT TAG AAT-3' (SEQ ID NO:7) and of 338 bp (PCR3; primer 5: 5'-AAT GCC TTT AAT AAT CTT GAT AGA AAT-3' (SEQ ID NO:8) & primer 6: 5'-CCC CCC GGG CAT ATG TCA TGA ACA TAT CAA TCT GTT TAA TC-3' (SEQ ID NO:9), and each fragment was sequentially cloned into pBluescript KS+ to produce plasmid pBS-TTC. The upstream primer 1 contained the Ribosome Binding Site (RBS) and translation initiation signals.

FIG. 2



PCR fragment NdeI/XhoI replacement: stop was replaced by NcoI site at the end of lacZ (amplification with primers GAL1/GAL2 between NdeI and XhoI sites)

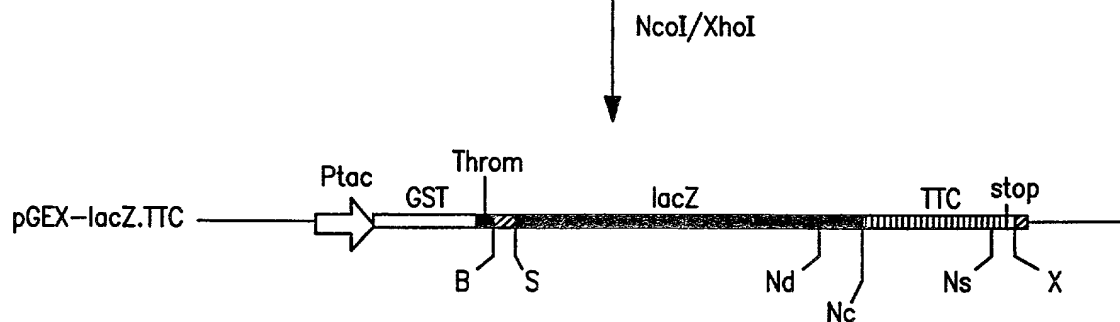
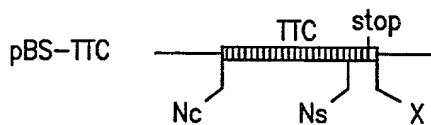
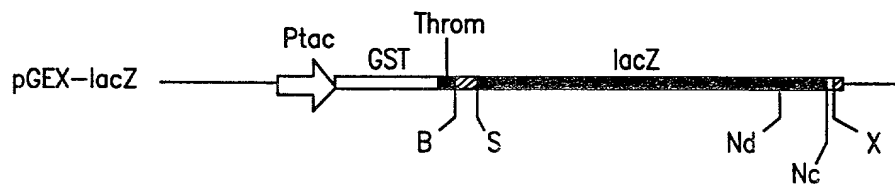
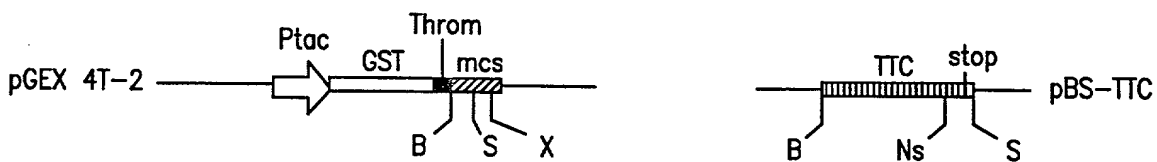
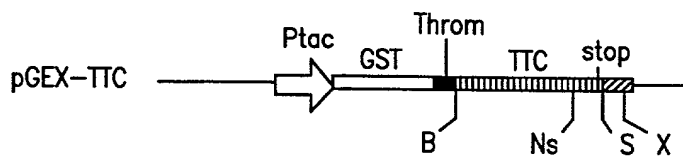


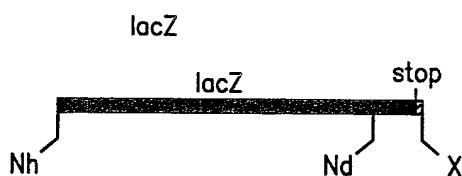
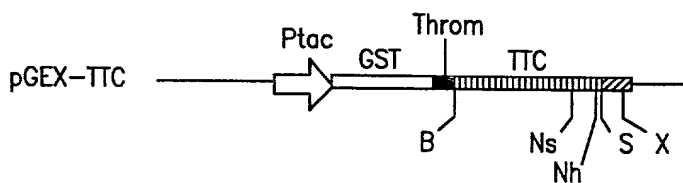
FIG. 3



BamHI/SmaI



PCR fragment NsiI/SmaI replacement: stop was replaced by NheI site at the end of TTC (amplification with primers TTC1/TTC2 between NsiI and SmaI sites)



NheI/XhoI

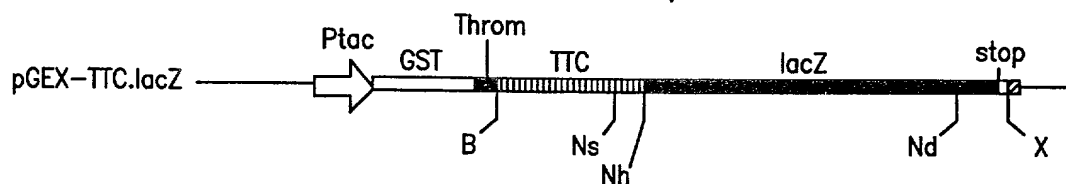


FIG. 4

pCMV.LACZ.TTC -> Graphic Map
 DNA sequence 8519 b.p. tagttattaata ... accgccatgcat circular

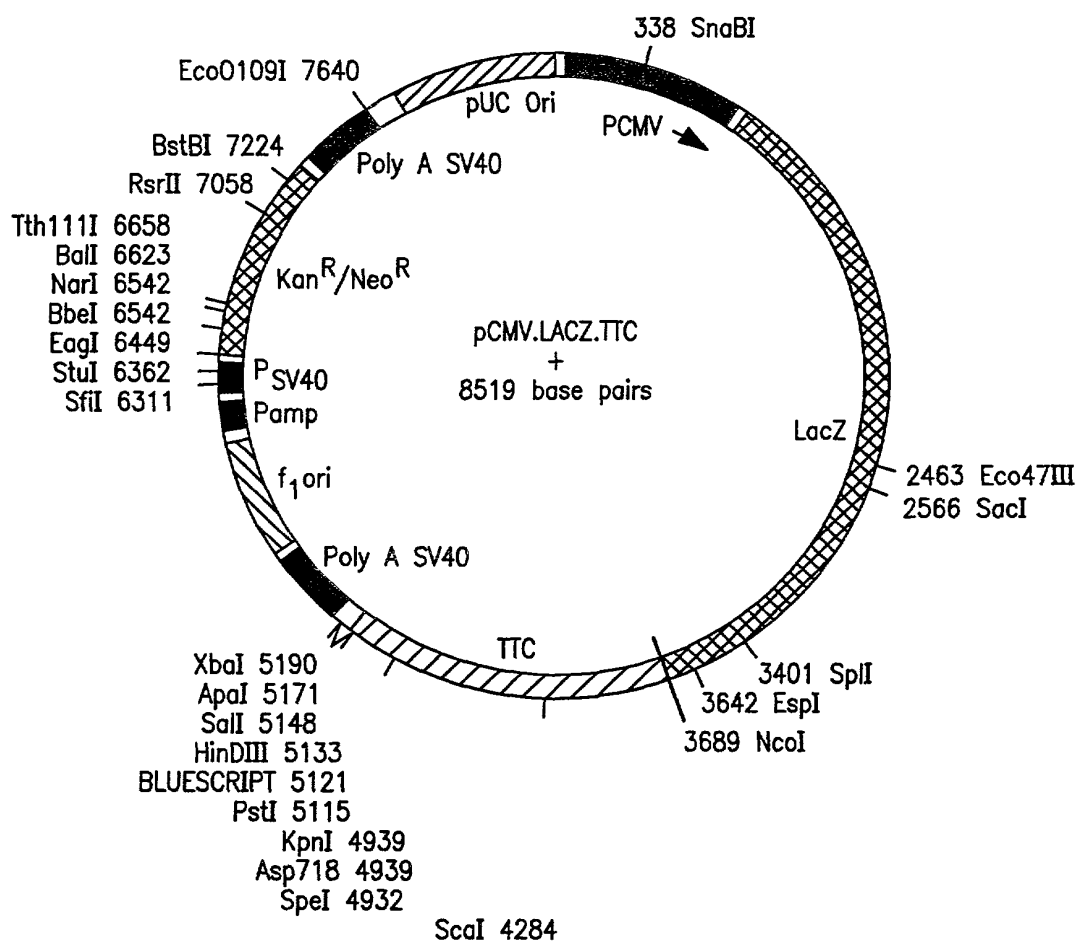


FIG. 5

09816467.062901